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(54) Title: METHODS OF PREPARING AMPLIFIED NUCLEIC ACID MOLECULES

(57) Abstract: New and improved methods are provided for generating amplified nucleic acid molecules from cellular mRNA. The methods are robust and reliable, and can be used to provide gene fragments for use in methods of analyzing gene expression patterns. The methods comprise: (a) preparing double-stranded cDNA by: (i) hybridizing at least one primer comprising an RNA polymerase promoter to said population of RNA molecules and extending said primer by reverse transcription to generate single-stranded cDNA, and (ii) synthesizing double-stranded cDNA from said single-stranded cDNA by priming with an oligonucleotide mixture having a random sequence selected from the group consisting of a tetramer oligonucleotide mixture, a pentamer oligonucleotide mixture, a hexamer oligonucleotide mixture, a heptamer oligonucleotide mixture, an octamer oligonucleotide mixture, a nonamer oligonucleotide mixture, a decamer oligonucleotide mixture and mixtures thereof; and (b) transcribing amplified copies of anti-sense RNA from said double-stranded cDNA.

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## INTERNATIONAL SEARCH REPORT

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**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC 7 C12Q1/68 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
 IPC 7 C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, PAJ, MEDLINE, SEQUENCE SEARCH

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	US 5 891 636 A (BARCHAS JACK D ET AL) 6 April 1999 (1999-04-06) the whole document	1-20
Y	US 5 851 805 A (MCCORMICK JOSEPH JUSTIN ET AL) 22 December 1998 (1998-12-22) column 10	1-20
A	US 5 665 547 A (LIANG PENG ET AL) 9 September 1997 (1997-09-09) the whole document	1-20
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Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 27317 A (CHEE MARK ;LAI CHAOQIANG (US); LEE DANNY (US); AFFYMETRIX INC (US)) 31 July 1997 (1997-07-31) example 14 -----	1-20
A	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1989 FRIEMERT C ET AL: "PREPARATION OF RADIOLABELED COMPLEMENTARY DNA PROBES WITH HIGH SPECIFIC ACTIVITY FOR RAPID SCREENING OF GENE EXPRESSION" Database accession no. PREV199192135875 XP002209828 abstract & METHODS IN MOLECULAR AND CELLULAR BIOLOGY, vol. 1, no. 4, 1989, pages 143-154, ISSN: 0898-7750 -----	1-20

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